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Müller, Christian P ; Quednow, Boris B ; Lourdusamy, Anbarasu ; Kornhuber, Johannes ; Schumann, Gunter ; Giese, K Peter

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Review - TiPS

CaM kinases - From memories to addiction

Christian P. Müller[1,2], Boris B. Quednow[3], Anbarasu Lourdusamy[4], Johannes Kornhuber[1], Gunter Schumann[2], K. Peter Giese[5]

[1] Department of Psychiatry and Psychotherapy, University Hospital, Friedrich-Alexander-University Erlangen-Nuremberg, Schwabachanlage 6, 91054 Erlangen, Germany.
[2] MRC Social, Genetic and Developmental Psychiatry Research Centre, Institute of Psychiatry, King's College London, De Crespigny Park, London SE5 8AF, UK.
[3] Experimental and Clinical Pharmacopsychology, Psychiatric Hospital of the University of Zurich, Lenggstrasse 31, CH-8032 Zurich, Switzerland
[4] Division of Child Health, Obstetrics and Gynecology, School of Medicine, University of Nottingham, NG7 2UH, UK.
[5] Centre for the Cellular Basis of Behavior, Institute of Psychiatry, King's College London, James Black Centre, 125 Coldharbour Lane, London SE5 8AF, UK.

Corresponding Author: Christian P. Müller, PhD
Section of Addiction Medicine
Department of Psychiatry and Psychotherapy
Friedrich-Alexander-University of Erlangen-Nuremberg
Schwabachanlage 6
91054 Erlangen, Germany
Phone: +49 (0) 9131 85 36896
Fax: +49-(0)9131-85-36002
Email: Christian.Mueller@uk-erlangen.de

Abstract

Drug addiction is a major psychiatric disorder with a neurobiological base that is still insufficiently understood. Initially, non-addicted, controlled drug consumption and drug instrumentalization are established. They comprise highly systematic behaviours acquired by learning and the establishment of drug memories. Ca^{2+}/calmodulin-dependent protein kinases (CaMKs) are important Ca^{2+}-sensors translating glutamatergic activation into synaptic plasticity during learning and memory formation. Here we review the role of CaMKs in the establishment of drug-related behaviours in animal models and in humans. Converging evidence shows now that CaMKs are a crucial mechanism of how addictive drugs induce synaptic plasticity and establish various types of drug memories. Thereby, CaMKs are not only molecular relays for glutamatergic activity; they also directly control dopaminergic and serotonergic activity in the mesolimbic reward system. They can now be considered as major molecular pathways translating normal memory formation into establishment of drug memories and possibly transition to drug addiction.

Keywords: Ca^{2+}/calmodulin-dependent protein kinase, drug dependence, addiction, psychostimulants, alcohol, opioids

Drug Use and Addiction

Drug addiction is a major psychiatric disorder for which only limited therapies are currently available [1,2]. A striking criterion for drug abuse and addiction is that it severely threatens one's own and others well-being and health. As such, there is a persistent need to treat drug addiction effectively, and ideally reverse the behavioural repertoire of an affected individual back to normal.

An important feature of drug addiction is that it develops from a behavioural repertoire, which is considered to be normal in many societies of the world: the controlled consumption and instrumentalization of psychoactive drugs [3,4]. Establishing and maintaining controlled drug consumption is based on systematic learning and memory retrieval of distinct behaviours, related to drug seeking, preparation, and consumption in a specific context [5,6]. Thereby, information is encoded within different behavioural systems, which can be summarized as "drug memories" [5,7-9] (Box 1). It is believed that an intensification of these memories together with a loss of impulse control (compulsivity) is responsible for the transition from controlled drug use to addiction [10-12]. Understanding how these drug memories are established and how they may take control over a normal behavioural repertoire should, therefore, allow to improve the prevention of addiction and to develop new and more effective treatments [13].

From Memories to Drug Memories

It was suggested that anatomical pathways, micro-morphological adaptations, as well as molecular mechanisms in the brain, overlap between normal learning and memory and drug memories [14-16]. A crucial player for memory formation in the brain is the glutamatergic system and its plasticity based on experience [17]. Addiction research considered glutamate as an important mediator during the establishment of drug use,

but also during escalation of consumption and addictive states [18]. The most abundant excitatory neurotransmitter in the brain, glutamate, activates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors to generate intracellular Ca^{2+}-transients. This cascade is pivotal for long-term potentiation (LTP) and subsequent alterations in gene expression that are the base for morphological adaptations at the synapse during learning [17]. A major pathway for this is the Ca^{2+} activation of calmodulin (CaM) and subsequent activation of Ca^{2+}/CaM dependent kinases (CaMKs). Here, we review how this memory pathway controls the establishment of drug-addiction related behaviours. In order to determine whether this role is drug specific or works as a common principle, CaMK function for different drug classes is compared.

Molecular Neurobiology of CaMKs

Calcium signalling through NMDA receptors is a fundamental step for inducing long-lasting synaptic plasticity, which is thought to be a key mechanism underlying learning and memory [19]. Due to high levels of CaM at the synapse, Ca^{2+} influx through NMDA receptors leads to formation of Ca^{2+}/CaM complexes [20], which activate CaMKs. Among the CaMKs, CaMKII has received most of the attention since this protein is very abundant in the brain [21] and because this multifunctional kinase has remarkable biochemical properties [22]. CaMKII is a holoenzyme that consists of 12 subunits [23]. αCaMKII and βCaMKII are the most abundant CaMKII subunits in the brain [21]. While αCaMKII is expressed only in glutamatergic neurons [24], βCaMKII occurs in inhibitory [25] and in excitatory neurons [26]. βCaMKII, but not αCaMKII, binds to F-actin. This localizes the CaMKII holoenzyme. The binding to F-actin is relieved by binding of Ca^{2+}/CaM [22]. The dissociation of CaMKII from F-actin

is thought to regulate actin polymerization that shapes synapse morphology [27]. αCaMKII, but not βCaMKII activity is critical for CaMKII function [26]. αCaMKII activity is primarily regulated by autophosphorylation at threonine-286 (T286), which results from an intersubunit kinase reaction within the holoenzyme. T286-autophosphorylation switches the kinase from Ca^{2+}/CaM-dependence to independence [28]. Currently, it is thought that T286-autophosphorylated αCaMKII prolongs CaMKII activity at the synapse after the Ca^{2+} stimulus [29]. This leads to glutamate receptor trafficking to the postsynaptic density, resulting in enhanced synaptic transmission [20]. Moreover, CaMKII activity is also regulated by 'inhibitory' autophosphorylation at T305/306 [30], endogenous CaMKII inhibitor proteins [31], and phosphatase activity [32]. Given its somewhat outstanding role in learning and memory, most addiction-related research has focused on CaMKII, which also puts it into the focus of this review.

In addition to regulation of CaMKII, Ca^{2+}/CaM also activates other members of the CaM kinase cascade. CaMKI, CaMKIV and Ca^{2+}/CaM kinase kinase (CaMKK) belong to this kinase cascade. CaMKK phosphorylates CaMKI and CaMKIV to activate these kinases [33]. This kinase cascade may have proof reading character in that only 'real' strong Ca^{2+} signals can induce it. Once activated, CaMKI and CaMKIV regulate gene transcription in the nucleus and local protein synthesis, which are needed for long-lasting synaptic plasticity [22].

CaMKs and Their Role in Memories

Mouse molecular genetic manipulations have now established that the CaMKs have a fundamental function in hippocampus-dependent learning and memory [22,34]. Loss of αCaMKII and βCaMKII expression impairs contextual and spatial memory

formation [26,34,35]. A point mutation that prevents the T286-autophosphorylation of αCaMKII severely impairs spatial and contextual memory formation [36,37]. In addition to having a role in memory formation, the T286-autophosphorylation has also been suggested to mediate memory storage and maintenance [20]. However, in the absence of T286-autophosphorylation, hippocampus-dependent long-term memory can still be formed after massed training [38]. Thus, CaMKII activity appears to be primarily important for the acceleration of memory formation but not for memory storage *per se* [39].

Members of the CaMK cascade are important for consolidation of hippocampus-dependent long-term memory. However, each member of this kinase cascade appears to have a specific role in consolidation rather than being important for all types of hippocampal memory consolidations. Accordingly, CaMKIV is required for consolidation of contextual, but not spatial memory [40,41]. Further, the β-isoform of CaMKK is likely to contribute to consolidation of spatial memory and memory of the delay version of the social transmission of food preferences task [42]. Interestingly, it is only required for this long-term memory formation in males, but not in females [43], thus, suggesting memory consolidation mechanisms differ between the sexes [44]. Similarly, αCaMKK is required for contextual, but not spatial, long-term memory formation in males, but not in females [45,46]. Genetic studies now support an important role of CaMKs also in human learning and memory (Box 2).

CaMKs and Their Role in Drug Memories

Distinct types of drug memories have been identified and grouped according to the system for normal memories (i.e. non-drug related memories) in humans (Box 1). Animal models of drug use and addiction, however, depict complex behaviours,

which normally involve several types of drug memories (Glossary) [47]. Neurobiological mechanisms may, therefore, overlap between single types of drug memories [5-8].

Psychostimulants

Psychostimulants drugs have common behavioural and subjective effects. They increase behavioural activity and arousal, and can also induce euphoria in humans. Drugs that share these effects include cocaine and amphetamine (AMPH) together with its derivatives [1]. An acute injection of cocaine [48] or AMPH [49] induces an increase in T286-phosphorylated αCaMKII levels in the striatum of rats, but has no effect on total αCaMKII levels (Figure 1). Chronic cocaine or AMPH administration and self-administration increases levels of αCaMKII in the nucleus accumbens (NAc) shell, but not NAc core of rats. This effect is paralleled by phosphorylation of the GluA1 AMPA receptor subunit and persists several weeks after drug administration (Table 1) [50-52]. An increase in αCaMKII activity leads to an upregulation of functional AMPA receptors [53] and receptor conductance [54]. After seven days of withdrawal from repeated cocaine administration, but not after fewer days, levels of CaMKII and phosphorylated CaMKII (pCaMKII) are increased in the NAc, prefrontal cortex (PFC) or dorsal striatum (DS). Both return to control levels several weeks after withdrawal [55-57]. The reinstatement of cocaine-seeking by a priming injection of cocaine induces an increase in pCaMKII in the NAc shell, but not in the NAc core of rats [58].

Locomotor Sensitization: Psychostimulant enhanced Ca^{2+}-influx through AMPA, NMDA, and L-type Ca^{2+}-channels leads to activation of CaM in dopaminergic neurons of the ventral tegmental area (VTA). Subsequent activation of CaMKII

enhances phosphorylation of various targets and promotes sensitization of hyperlocomotor responses in a context-dependent way [59] (Table 1, Glossary). CaMKII activation in the VTA, NAc, and hippocampus appears of crucial importance for locomotor sensitization to cocaine and AMPH [60-64]. The sensitization of the AMPH-induced locomotor activation is blunted in αCaMKII deficient mice [65]. Transient inhibition of αCaMKII in the NAc shell reverses the increase in pGluA1 levels and attenuates AMPH-induced locomotor sensitization [66]. αCaMKII autophosphorylation-deficient mice have preserved sensitization of the hyperlocomotor effects of cocaine [67]. Mice which lack CaMKIV in dopamine D1-receptor expressing neurons do not sensitize to the hyperlocomotor effects of cocaine [68].

Conditioned place preference (CPP): CaMKII activation in the VTA, NAc, and hippocampus appears of crucial importance for CPP establishment after cocaine and AMPH treatment [61-64]. αCaMKII-deficient mice are not impaired in AMPH- or cocaine-induced CPP when only endpoints of the learning are considered [65]. However, αCaMKII autophosphorylation-deficient mice are significantly delayed in their ability to establish cocaine-induced CPP [67]. Mice with a striatum-specific autonomously active CaMKII show potentiated cocaine CPP after low, but not after high doses of cocaine [64]. Several studies identified the VTA-NAc axis as a crucial pathway of CaMKII action in the establishment of cocaine-induced synaptic plasticity and CPP [69,70]. Local injection of the CaMKII inhibitory peptide TatCN21 into the VTA blocks establishment of cocaine-CPP and cocaine-evoked depression of excitatory synaptic transmission in the NAc shell [70].

Mice lacking CaMKIV in D1-receptor expressing neurons show enhanced establishment and reinstatement of cocaine-CPP [68]. However, reduced CaMKIV activity is also associated with enhanced social anxiety [71] and reduced fear

learning [41]. These findings suggest that CaMKIV may work as a resilience factor against cocaine addiction possibly by stabilizing emotional tone.

Self-administration: The activation of CaMKII in the VTA, NAc, and hippocampus appears of crucial importance for self-administration of cocaine and AMPH [61-64]. Transient overexpression of αCaMKII in the NAc shell increases not only pGluA1 levels, but also AMPH self-administration in rats [72]. Transient inhibition of αCaMKII in NAc shell reduces AMPH self-administration in sensitized rats [51,66]. Cocaine effects on αCaMKII-, but not βCaMKII expression are suggested to be essential for the motivation to self-administer cocaine. αCaMKII expression correlates with break points during cocaine self-administration. In turn, virus-mediated down-regulation of αCaMKII expression in the NAc shell reduced break points [73]. In the NAc shell, αCaMKII binds directly to D3-receptors. This binding is Ca^{2+}-sensitive and can be enhanced by autophosphorylation of αCaMKII. Recruitment of αCaMKII to D3 receptors transiently inhibits receptor efficacy [74] (Table 1). Downstream αCaMKII action, reduced D3 receptor function at the level of the NAc was associated with enhanced impulsivity and cocaine reinforcement [10], which may facilitate the transition to compulsive psychostimulant self-administration [11]. While there is no direct evidence that CaMK-dependent drug memories override normal CaMK-dependent memories, as it would be expected in the time course of addition establishment, indirect evidence suggests at least a competition at the level of CaMK-induced morphological changes. It was shown that AMPH self-administration, which leads to dendritic arborization and an increase in spine density in the neocortex and NAc, would prevent morphological changes induced by normal learning [75,76]. Whether CaMK-dependent drug memories are stronger than normal memories and, therefore, induce a shift in behavioral repertoire from normal to

addiction-related behaviors, is currently unclear. Other factors, like the loss of impulse control over established drug-related behaviors, are under serotonergic control [12], which is, in turn, CaMK dependent [129,132].

Reinstatement of self-administration: The reinstatement of cocaine seeking by D1-receptor activation in the NAc shell is mediated by L-type Ca^{2+}-channels and subsequent phosphorylation of αCaMKII at T286 and of AMPA GluA1- subunits (Table 1). Blocking CaMKII in the NAc shell, but not in the basolateral amygdala (BLA), attenuates cocaine-induced reinstatement of cocaine-seeking [58,77]. This suggests a strong link between dopaminergic and glutamatergic signalling in the reinstatement of cocaine-seeking mediated by CaMKII [58].

Dopamine activity: The mesocorticolimbic dopamine system plays a crucial role in the establishment of psychostimulant abuse-related behaviors [1]. Basal dopamine level in the DS of αCaMKII deficient mice are significantly enhanced compared to wild-type (WT) mice [65]. This is not due to an altered dopamine synthesis rate or enhanced dopamine transporter (DAT) -mediated dopamine uptake [65,78]. However, enhanced vesicular dopamine release is found in αCaMKII deficient mice, which may explain the enhanced extracellular dopamine levels [65].

The AMPH-induced increase in extracellular dopamine levels is markedly reduced in striatal synaptosomes, in brain slices, and *in-vivo* in αCaMKII deficient mice. This is in line with findings showing αCaMKII and its T286 autophosphorylation to be essential requirements for AMPH-triggered substrate efflux at the DAT [65,78]. Withdrawal from repeated cocaine- or AMPH treatment sensitized striatal dopamine responses to an AMPH challenge in rats. This sensitization could be blocked with the rather unspecific CaMKII inhibitor KN-93 [79,80], suggesting a role of CaMKII in neurochemical sensitization during chronic psychostimulant exposure.

The lack of αCaMKII autophosphorylation enhances basal dopamine levels in the NAc and PFC of mice. The cocaine-induced dopamine increase in both brain regions, however, is attenuated. Reduced neurotransmitter activation is associated with lower c-Fos activation, as an indicator of reduced cellular activation, after cocaine in the NAc and hippocampus [67,81]. CaMKII phosphorylates tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis, and the DAT [82,83]. Since TH, DAT, CaM, and CaMKII are co-expressed in mesolimbic projections [78,84], it has been suggested that dopamine synthesis is regulated by CaMKII-controlled TH activity [85]. An increase in basal dopamine levels may, thus, limit the capacity for the drug-induced dopamine increase. The αCaMKII mediated DAT-phosphorylation is essential for an AMPH-induced dopamine increase, but not for regulation of basal dopamine efflux [78,84]. The DAT is a binding partner of αCaMKII in dopaminergic neurons. CaMKII has a strong influence on dopaminergic activity. However, there is also some feedback, in that D2-, D3-, and D4 receptors regulate CaMKII activity [74,86-88]. It should be noted that CaMKs interact also with other neurotransmitter systems which are pivotal for drug-related behaviors (Table 1).

Serotonin activity: In the absence of a dopamine response, rewarding effects of psychoactive drugs can be mediated by the serotonergic system [12,83]. The lack of αCaMKII autophosphorylation enhanced basal serotonin (5-HT) levels in the NAc and PFC of mice. In contrast, the cocaine-induced 5-HT increase in both brain regions was abolished in the αCaMKII^{T286A} mice [67]. Tryptophan hydroxylase 2 (TPH2) is the rate-limiting enzyme in the biosynthesis of 5-HT in the brain. Its activity is under the control of CaMKII [126]. A lack of αCaMKII autophosphorylation may limit the capacity of transmitter available for release into the extracellular space upon excitation. This may lead to reduced 5-HT activation, which may account for the delay in the establishment of the rewarding effects of cocaine [12,67]. αCaMKII binds

to serotonin transporters (SERT) in neurons, but with lower affinity than to the DAT. Pharmacological inhibition of CaMKII impairs the AMPH-induced SERT efflux, but not substrate uptake [127]. While CaMKII has a strong influence on serotonergic activity, there is also a feedback regulation of CaMKII activity by 5-HT_{1A}-receptors [128,129].

Opioids

Activation of opioid receptors induces a Ca^{2+}-transient which also activates CaMKs [90] (Figure 1). μ-Opioid receptors, which are co-localized with CaMKII in neurons [91], are also targets for CaMKII [92] (Table 1). The CaMKII mediated phosphorylation of μ-opioid receptors contributes to functional desensitization after activation [93]. An acute morphine application enhances autophosphorylation and activity of αCaMKII in the striatum, hippocampus, and PFC of rats [94-97], without affecting αCaMKII gene expression [98]. Chronic morphine administration during CPP learning increases CaMKII and pCaMKII in the ventral- and dorsal striatum as well as in the hippocampus of rats [97,99]. This is paralleled by an increase in αCaMKII mRNA expression [98]. Chronic morphine treatment increases expression of CaMKIV in the CA3 region of the hippocampus of mice, whilst it decreases it in the DS and other brain areas [100]. Withdrawal from repeated morphine and heroin administration, or naloxone precipitated withdrawal increases CaMKII activity and mRNA levels in the hippocampus and medial PFC (mPFC) of rats [94,98,101,102]. Heroin withdrawal has no effects on βCaMKII or CaMKIV levels in the mPFC [102], while CaMKII activity returned to control levels within a week of withdrawal [16]. The reinstatement of morphine self-administration by a priming injection of morphine induces an increase in T286 p-αCaMKII, but not p-βCaMKII levels in the NAc shell of rats compared to levels during extinction [103].

CPP: The establishment of morphine-induced CPP depends on CaMKII in the brain [99]. Pharmacological inhibition of CaMKII in the hippocampus and amygdala blocks the establishment and reinstatement of morphine-induced CPP [104]. Pharmacological inhibition of CaMKII in the mPFC, but not in the BLA, blocks the establishment of morphine- and heroin-induced CPP in naïve rats. In contrast, in chronically treated and withdrawn rats, mPFC blockade of CaMKII has no effect on CPP-establishment. Intra-BLA pharmacological blockade of CaMKII, however, blocks morphine- and heroin CPP-establishment [102,105]. This effect is most evident in the late phase of CPP consolidation 12h post conditioning [106]. CaMKIV is required for the establishment of morphine-induced CPP in mice, since CaMKIV KO mice show a significantly reduced CPP for morphine [107].

Self-administration: Inhibition of CaMKII activity in the NAc shell reduces the reinstatement of morphine-seeking in rats [70,103]. This effect is potentially mediated by a decrease in T286 αCaMKII phosphorylation in the NAc [70].

Withdrawal and abstinence: Withdrawal from chronic morphine administration induces withdrawal symptoms. The development of withdrawal symptoms is blocked by intra-hippocampal injection of the unspecific CaMKII inhibitor KN-62 [92]. After seven days of morphine treatment, naloxone precipitates withdrawal symptoms, like wet-dog shakes, paw tremors, or jumping. There is no difference in these withdrawal signs between CaMKIV KO and WT mice, which suggests no role for CaMKIV in morphine withdrawal behaviors [107].

Alcohol

Acute alcohol application does not affect CaMKII activity in astrocytes [108]. In contrast, repeated alcohol administration enhances CaMKII activity in neurons and astrocytes (Figure 1) [108,109] and increases phosphorylation rate [110]. Free choice

alcohol drinking increased protein levels of αCaMKII, but not βCaMKII in the amygdala and NAc of mice [111]. Operant alcohol self-administration increases p-αCaMKII levels in the amygdala without changing total αCaMKII levels [111].

CPP: CPP measures the learning and expression of an association between the incentive properties of the drug with environmental cues. Altered CPP may, thus, result from changes in learning and memory or/and changes in the rewarding value of a drug [47]. αCaMKII autophosphorylation plays a special role in the speed at which alcohol induces CPP in mice. Preference for an alcohol-paired environment was established after seven conditioning trials in WT mice. Surprisingly, αCaMKII autophosphorylation deficient mice (αCaMKII^{T286A}) establish a profound alcohol CPP after only a single alcohol conditioning trial [112]. The disparity between the reported establishment of alcohol drinking [113] and CPP [112] may be explained by the acute effects of alcohol in the CPP paradigm. CPP involves several learning processes and is not simply a result of incentive-driven behaviour [47]. αCaMKII^{T286A} mice display enhanced activity and hyper-arousal in response to potentially threatening environments, while showing no altered behaviour in well-habituated environments [114]. Arguably, one key component to compulsive drug seeking is the alleviation of a negative affective state resulting in negative reinforcement [115]. Alcohol drinking behaviour was measured in a familiar home cage environment while CPP rewarding effects were measured in a less familiar environment, which might together with the injection process represent an aversive/threatening stimulus. This view is supported by the observed hyperactivity in αCaMKII^{T286A} mice during CPP baseline and novel open field exposure, but not in familiar home cages [114]. In contrast to WT mice, alcohol has an acute and persistent sedating effect in the αCaMKII^{T286A} mice. One possible explanation of this effect is that alcohol alleviated the threat-induced

behavioural responses in αCaMKII^{T286A} mice in a potentially aversive new test situation, thus driving CPP learning [47].

Self-administration: CaMKII activity and autophosphorylation in the amygdala is required for operant alcohol self-administration as local pharmacological inhibition of both decreased alcohol-reinforced responding [111]. αCaMKII^{T286A} mice showed initially reduced alcohol drinking in a two-bottle free-choice procedure. This effect is persistent until animals are withdrawn and reinstated twice to alcohol [113]. These findings suggest that αCaMKII autophosphorylation controls the speed at which alcohol drinking is established, in a similar way as it may control the establishment of non-drug related learning [28].

Dopamine activity: There is a significantly reduced dopamine response to alcohol in the NAc of αCaMKII^{T286A} mice [113]. An analysis of the cellular activation of the VTA, as an origin of the mesolimbic dopamine projections, revealed an enhanced activation after acute and subchronic alcohol exposure in the rostral, but not caudal VTA in αCaMKII^{T286A} mice, which appears to be driven predominantly by GABAergic neurons [113]. Also cellular activation after alcohol in the hippocampus is reduced in αCaMKII^{T286A} mice [81].

Serotonin activity: The lack of αCaMKII autophosphorylation provoked an alcohol-induced 5-HT increase in the PFC which was not observed in WT animals [113]. High alcohol drinking has been associated with deficiencies in brain 5-HT levels and turnover [130]. An induced 5-HT increase, in turn, can reduce alcohol drinking [131]. The alcohol-induced 5-HT increases in the PFC of αCaMKII^{T286A} mice may, therefore, contribute to the reduced alcohol drinking seen in these mice. There is a strong link between CaMKII and serotonergic function in the brain. CaMKII is required for the phosphorylation and activation of TPH, the rate limiting enzyme in the biosynthesis of 5-HT [126]. Pharmacological inhibition of CaMKII has been shown to increase the

firing rate of 5-HT neurons [132]. This supports the view that a reduced CaMKII function may increase 5-HT neuronal firing and terminal 5-HT release, which then inhibits alcohol consumption. These data may suggest that αCaMKII autophosphorylation controls the speed at which an alcohol preference is established, but not the capacity to consume alcohol, at least in part, by a serotonergic mechanism.

Nicotine

Acute subcutaneous administration of nicotine increases activity of CaMKII in the VTA, NAc, and amygdala in mice. Nicotine acute effects on CaMKII activity mainly depend on β2 acetylcholine receptor subunits, but less on α7 subunits [116]. It was also suggested that the nicotine-induced β2 mediated phosphorylation of cAMP response-element binding protein (CREB), which is required for the rewarding properties of nicotine [117,118], is mediated by CaMKII activation in the VTA and NAc [116]. CaMKII appears to play a role in nicotine withdrawal behaviours. Pharmacological inhibition of CaMKII reduced somatic nicotine withdrawal signs, but enhanced anxiety [119].

Concluding remarks

CaMKs are abundant proteins in the brain. In neurons they are a crucial transducer of Ca^{2+}-transients into the activation of a plethora of functional targets involved in acute molecular and long-term plastic changes at synapses that are the base for learning and memory. Accumulating research has now provided evidence for the view that the same CaMKs serve also acute and long-term molecular plasticity that directly supports the various types of drug memories. Causal chains are now emerging: Many (if not all) addictive drugs increase CaMK activity, foremost that of αCaMKII, by

either enhanced expression, enhanced phosphorylation, or both. This increase lasts during periods of drug intake and is a necessary prerequisite for the learning and consolidation of numerous drug-related behaviours. During withdrawal, CaMK activity usually declines with some delay, just to rise again during reinstatement of drug-seeking and consumption. Also this behaviour is facilitated by a boost of CaMK activity. Various studies have now identified binding partners and phosphorylation targets of CaMKs. These comprise not only glutamatergic receptors. Important evidence has now demonstrated an extensive control of dopaminergic and serotonergic activity in the mesolimbic system, which are crucial mediators of drug reward. CaMK activation of these targets may, thus, provide a large number of parallel functional pathways by which a drug-induced Ca^{2+}-transient may control drug memory establishment and consolidation and, eventually, pave the way to drug addiction. This would suggest CaMKs as excellent pharmacological targets to prevent the establishment of drug memories. However, there are obstacles that currently limit this possibility. CaMK-dependent drug memories compete with normal memories and use the same downstream pathways. Currently, there is no CaMK, which is exclusively activated by drugs, but not during normal memory formation. One way to circumvent this problem could be the tailoring of CaMK-based pharmacotherapies to settings and time points where little normal learning is required and where drug memories can specifically activated and pharmacologically attenuated (see: Outstanding questions). Another future perspective would be the development of subtype specific ligands that can target major CaMKs more selectively and spare others that are primarily involved in other behaviours [120].

Box 1: Drug memories and related preclinical tests

Parallel to non-drug related memory systems [121], two major drug-memory categories can be distinguished: a *declarative drug memory* and a *non-declarative drug memory* [5-8]. The declarative drug memory should comprise a semantic memory for drug facts and a memory for drug episodes. The *semantic memory* for drugs contains all impersonal facts, rules, and concepts involving drugs, e.g., their names, where they come from, recommended doses, what others report about its effects, and the social rules of their consumption. The *episodic drug memory* comprises the memories of personally experienced episodes with the drug. It is an autobiographical memory on the 'what', 'where', and 'when' of the personal drug encounters. This may include memories of subjectively experienced acute drug effects, e.g., the mental states the drug induced [9].

The non-declarative drug memory contains engrams of the classically conditioned drug memory, instrumentally conditioned drug memory, habit memory, procedural drug memories, and drug priming memories [8]. *Classically conditioned drug memories* may contain all drug effects that refer to the process of Pavlovian conditioning. These may include e.g., the sensitization of the acute locomotor effects, drug tolerance, partially conditioned place preference, conditioned locomotor activity, conditioned emotional and physiological responses, and conditioned withdrawal effects. *Instrumentally conditioned drug memories* comprise engrams established by instrumental conditioning. Major behaviours induced by these engrams are drug-seeking behaviours and drug self-administration. These memories also include drug-cues which can serve as secondary reinforcers, as in conditioned place preference [47], or which can reinstate drug-seeking and drug self-administration behaviour. *Drug habit memories* refer to instrumental behaviour that is no longer goal directed, but stimulus controlled and independent from its behavioural consequences. This

type of memory plays an important role in the transition from controlled to compulsive drug use and addiction. *Procedural drug memories* comprise all memories for skills involved in handling a drug. This may range from its production (e.g., cooking up heroin) to the method of self-administration (e.g., snorting cocaine). *Drug priming memories* refer to those engrams whose activation by a small amount of the drug, which would not induce major subjective and behavioural effects in drug naïve individuals, can induce drug-related behaviour (e.g., reinstate drug seeking, conditioned place preference, or self-administration) and subjective effects in experienced users.

Box 2: CaMKs shaping human memories and drug memories

Genetic association studies provide evidence for association of CaMKs with memory performance in humans. Easton and colleagues [122] provided the first evidence of αCaMKII involvement in human memory functions by demonstrating an association between CAMK2A gene single nucleotide polymorphisms (SNPs) and spatial as well as non-spatial working memory in two populations of young healthy subjects. In line with the association findings in healthy humans, two other CAMK2A SNPs have been recently associated with the risk of Alzheimer's disease even though in relative small samples [123].

In a translational effort, postmortem evidence and genetic associations of CaMK gene polymorphisms with various drug use- and addiction-related behaviors have been found. Increased NAc levels of CaMKII were reported for cocaine-dependent humans compared to controls in a postmortem study [52]. Recently, an association between the CAMK2A SNP, rs3776823, and the slope time by which severe cocaine consumption was established was found in two independent samples of Brazilian and Swiss cocaine users [67]. These findings suggest that polymorphisms in the CAMK2A gene may contribute to the speed of acquiring a severe level of cocaine intake once consumption has commenced. A genetic study with 670 cocaine dependent individuals and 726 controls from São Paulo, Brazil, revealed a significant association of cocaine abuse with the rs919334 SNP in the CaMKIV gene promotor region [68].

In agreement with animal data, several associations between SNPs in the human CAMK2A gene and alcohol use and dependence were found. Seven SNPs typed from the CAMK2A gene were found to be significantly associated with alcohol dependence [113]. Notably, the rs10463293 SNP has previously been associated with working memory performance [122], while SNP rs3756577 has been suggested

before to influence the risk for Alzheimer's disease [123]. A set of 13 CAMK2A SNPs were found to predict the number of alcohol drinking days per month in a sample of adults living in the US [124]. The SNP with the lowest p-value (rs7711562) was also significantly associated with alcohol dependence in the study by Easton et al. [113]. While emerging evidence revealed an important role of CaMKs in drug use behavior and addiction, it also showed crucial involvement in other psychiatric disorders which are frequently co-morbid with drug addiction [125]. These findings suggest a significant overlap between CaMK genetic mechanisms controlling normal learning and memory and those involved in drug memories in humans.

Glossary:

Behavioural paradigms to test drug use and addiction behaviours

Drug use and subsume numerous distinct behaviours. These behaviours can be investigated separately in animal models. The most important of them have been used to discover the role of CaMKs.

Locomotor sensitization: measures the progressive augmentation of the hyperlocomotor effects. Sensitization can be context dependent involving a classical conditioning or context independent which is assumed not to result from a learning process, but from a sensitization of neuronal circuits in the brain to the drug. The establishment and expression of sensitization is part of psychoactive drug addiction [7,8].

Conditioned place preference: measures the incentive properties of a drug by its conditioning to a spatial context (place in a box). Thereby, an increase in the time spend in a previously drug-paired environment indicates incentive properties of a drug. CPP learning and expression can be tested separately and involves both, classical and instrumental conditioning [47].

Self-administration: measures if and at which work load (e.g. bar pressing) animals learn to self-administer a drug. The route of self-administration differs between drugs, e.g. oral for alcohol and i.v. by an implanted catheter for other drugs. Rapid learning and a high motivation to work for the drug indicate strong reinforcing effects of the drug and, thus, a high potential for addiction. It is believed that instrumental conditioning is the major learning mechanism for operant drug self-administration [7,8].

Reinstatement of self-administration: measures the ability of a stimulus that was paired with the availability of the drug, to reinstate self-administration behaviour after a period when the drug was not available and the behaviour extinguished. Such a

stimulus can be sensory (e.g. a cue) or intrinsic (the effects of the drug itself). Reinstatement is believed to model in particular relapse behaviour [7,8].

Open questions

- Most research revealed an important facilitating role for αCaMKII in drug memory establishment. CaMKIV rather appears a resilience mechanism. What is the role of other CaMKs, such as CaMKI and CaMKK, in drug memories?
- αCaMKII is a crucial mediator of LTP, which has been shown to be an important mechanism in normal learning. Does αCaMKII also drive drug-induced LTP and is this LTP a necessary condition for distinct types of drug memories to be established?
- There is a dissociation in CaMKII activation between brain areas during different stages of the drug taking time line. Most evidence suggests the VTA and NAc shell as a crucial anatomical locus for drug memories. However, is there a functional impact of drug-induced CaMK activity changes in other brain areas, such as the hippocampus, amygdala, or PFC?
- Currently available CaMKII inhibitors are not specific for CaMKII. They also block other CaMKs and other molecules. However, many of the mechanistic findings rely on these inhibitors. Can findings be confirmed with more specific inhibitors?
- Research has focused mostly on cocaine, amphetamine, morphine, heroin, and alcohol. Do emerging principles of e.g., αCaMKII action also apply for other widely abused drugs, such as cannabis, γ-hydroxybutyrate, methamphetamine, or halluzinogenic drugs?
- The strong overlap in the function of CaMKs between normal learning and memory and drug memories makes it difficult to use them as plain pharmacological targets. However, would there be time windows, e.g., in therapeutic settings, when selective inhibition/stimulation of CaMKs could be beneficial for addiction therapy?

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Figure legends

Figure 1 αCaMKII activity during long-term repeated exposure to major addictive drugs and its functional consequences. A). Psychostimulants like cocaine or amphetamine increase activity of αCaMKII in the nucleus accumbens (Ncl. acc.) shell of the brain after acute and chronic psychostimulant administration (red arrows = single drug episodes). Thereby, an increase in αCaMKII activity can be induced by enhanced phosphorylation and/or expression. The activity increase is paralleled by phosphorylation of various targets, such as AMPA receptor GluA1 subunits, extracellular signal-regulated kinase-1, CREB and many more [48,49]. Interestingly, GluA1 Ser831-phosphorylation is one of the prerequisites for appetitive incentive learning [133]. Altogether, this appears to facilitate the establishment and expression of numerous drug-related behaviours (↑). After cessation of drug administration, αCaMKII activity declines to basal levels. During reinstatement of drug self-administration, however, it is increased again. Importantly, the action of αCaMKII appears brain area specific, no such response or facilitation of behaviour was found e.g., in the nucleus accumbens core. B.) Opioid drugs like morphine or heroin increase the activity of αCaMKII in the hippocampus and nucleus accumbens/ shell of the brain after chronic drug administration. This increase appears to facilitate the establishment of the rewarding effects of the drugs. After cessation of drug administration, αCaMKII activity remains elevated before it eventually declines to basal levels. This elevation was shown to contribute to the expression of withdrawal symptoms. During reinstatement of drug self-administration αCaMKII activity is increased again, which is required for this drug-related behaviour. Importantly, the action of αCaMKII appears brain area specific, no such response or facilitation of

behaviour was found e.g., in the nucleus accumbens core. C.) Alcohol increases the activity of αCaMKII in the amygdala and nucleus accumbens after chronic drug administration. Alcohol drinking also increased Ser831-phosphorylation of AMPA GluA1 subunits, which are co-expressed with αCaMKII in neurons [104]. These effects appear to facilitate the establishment of some drug-related behaviours (↑), but may also limit other behaviours (↓). αCaMKII activity during withdrawal and reinstatement and its contribution to alcohol-related behaviours at these time points is currently unclear and awaits further investigation.

Table 1 Major binding partners and phosphorylation targets of αCaMKII in the brain (↑ enhanced function, ↓ reduced function, -- no effect on function; * targeted by αCaMKII; AMPH - amphetamine, DA – dopamine).

Table 1

transmitter system	target	target function	phosphorylation site	effect on function	references
glutamate	**GluR1**	AMPA receptor subunit	Ser831	↑	134-139
	NR2B	NMDA receptor subunit	Ser1303	↑	140,141
dopamine	**tyrosine hydroxylase**	dopamine synthesis		↑	142-144
	dopamine transporter	dopamine clearance	N terminal serines*	↑ (AMPH-induced DA efflux)	65,78,85
	D3 receptor	dopamine receptor	3rd intracellular loop*	↓	74
serotonin	**tryptophan hydroxylase 2**	serotonin synthesis	Ser19	↑	145,146
	serotonin transporter	serotonin clearance	N terminal serines*	↑ (AMPH-induced 5-HT efflux) --- (substrate uptake)	127
endogenous opioides	**μ-opiate receptor**	opiate receptor	Ser261 and Ser266	↑ desensitization	147-149
GABA	**$GABA_A$ receptor**	GABA receptor	b2 subunit	↑	150
general	**L-type Ca^{2+} channel**	Ca^{2+} channel		↑	151
	BK channel	high conductance Ca^{2+} activated voltage gated K^+ channel	Thr107	↑	152
				↓	153
	SynGAP	Ras-GTPase activating protein		↓	154
	ΔFosB	transcription factor	Ser27*	↑	52

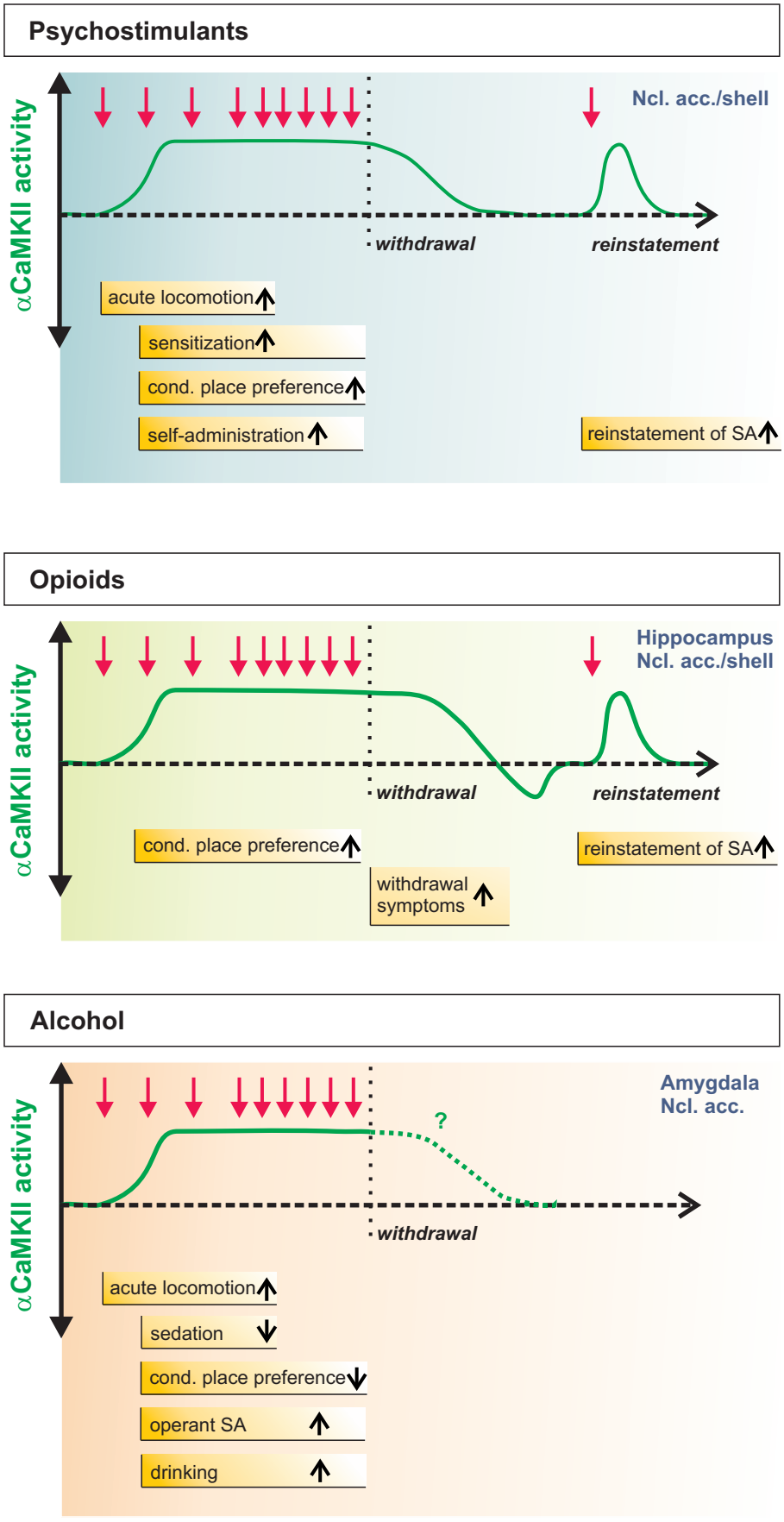

Figure 1